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Project Summary

Effects of Halogenated Aromatic Compounds on the Metabolism of Foreign Organic Compounds

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This work was initiated to extend our previous findings on the induction of xenobiotic metabolism by the halogenated benzenes. Particular interest was focused on studying further the relationship between their long-term induction and their storage in body fat. A second objective was to determine if the brominated benzenes caused hepatic porphyria, similar to that observed with the fungicide hexachlorobenzene. A third aim was to extend our observations to other halogenated aromatic compounds. The final objective was to examine the role of the halogenated benzenes in the enhancement of esteratic pathways of xenobiotic metabolism. Sprague-Dawley rats were used for all experiments involving animals, with the exception of Swiss-Webster mice (Laboratory Supply Co., Indianapolis, IN) being used to measure esterases and pesticide toxicity.

In contrast to hexachlorobenzene, the brominated benzenes, including the fully substituted hexabromobenzene, did not induce hepatic porphyria to any significant degree and did not cause increases in the excretion of porphyrins.

A number of trichlorophenols were assessed for their potential to increase xenobiotic metabolism. They did not increase any of the indicators of induction.

The brominated diphenyl esters, commercial mixtures used as fire retardants, were found to be potent inducers of xenobiotic metabolism. Administration of low doses for 90 days resulted in effects which were prolonged far beyond the period of administration. The chlorinated diphenyl ether isomers did not share this potent induction ability.

The chlorinated benzo-*p*-quinones examined were not found to be inducers, but did demonstrate a very strong cumulative toxicity.

Studies on the distribution and elimination of 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene indicated their prolonged inductive effects were related to storage and slow release, particularly in adipose tissue. Aroclor 1254 caused prolonged increases in xenobiotic metabolism, which could also be enhanced by starvation.

The halogenated benzenes induced esterases associated with the metabolism of acetanilide, procaine and phenyl acetate. They were able to protect against the lethality and inhibition of cholinesterase activity associated with the organophosphate insecticides parathion and malathion, plus their active metabolites, paraoxon and malaoxon. This protection was accomplished by enhancing the metabolic detoxification of these compounds via both esterases and mixed function oxidases.

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EP 1.89/2:

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, OH, to announce key findings of the research project which is fully documented in a separate report of the same title (see Project Report ordering information at back.)

Introduction

There is great interest in the induction of xenobiotic metabolism by halogenated aromatics, and the resultant interactions of these compounds with the actions of concomitantly administered chemicals or naturally occurring chemicals, such as hormones. Most studies, however, have focused on the polychlorinated biphenyls, polybrominated biphenyls, and chlorinated dibenzo-*p*-dioxins. Previous studies in our laboratory have indicated that the chlorinated and brominated benzenes are also inducers of xenobiotic metabolism.

There are definite structure-activity relationships, with brominated benzenes more active than chlorinated analogs. In general, the greater the degree of halogenation, the greater the degree of induction. Second, effects are prolonged beyond the period of administration of the halogenated benzenes.

Observations pertaining to the actions of halogenated benzenes on mixed function oxidases led to the necessity of extending the investigation to; actions on porphyria and esterase activity, examining the relationship between storage of halogenated benzenes and their long-term effects, and exploring the possibility that other halogenated aromatic compounds might also be inducers of xenobiotic metabolism.

Hepatic Porphyria

Hexachlorobenzene has been observed in a number of laboratory species and in humans to cause more porphyrinogenic effects than do the less chlorinated benzenes. Brominated compounds are often more active than chlorinated analogs. Therefore it became important to investigate the effects of the fire retardant hexabromobenzene (HBB) on porphyrin synthesis. In the present studies, the effects of two less halogenated benzenes, 1,4-dibromobenzene and 1,2,4-tribromobenzene, were also examined.

Halogenated Aromatics as Inducers of Xenobiotic Metabolism

Chlorinated benzenes induce microsomal enzymes associated with xenobiotic metabolism. Very little is known of the potential of chlorinated phenols. It appeared possible that the chlorinated benzenes may not be direct inducers of xenobiotic metabolism, but indirectly with conversion to chlorinated phenols. This study was undertaken to determine whether or not trichlorophenols could alter xenobiotic metabolism.

Brominated diphenyl ethers are structurally similar to several halogenated aromatic compounds known to induce xenobiotic metabolism. These compounds are commercial mixtures, making it imperative to compare them with diphenyl ether itself, bis(*p*-bromodiphenyl) ether, and the fully brominated diphenyl ether.

Chlorinated diphenyl ethers are contaminants of chlorinated phenols and readily concentrated, for example, by fish. Because of the structural-activity relationships among chlorinated biphenyls and chlorinated dioxins, the chlorinated diphenyl ethers were examined. Also, the potency of individual isomers were compared to commercial mixtures of brominated diphenyl ethers.

Benzoquinones were examined for effects as inducers. Although they did not qualify in this respect, they were found to have very cumulative toxicity. Benzofurans were very potent inducers of xenobiotic metabolism.

Relationship between Induction and Storage

Municipal wastewaters contain several chlorinated and brominated benzenes which induce xenobiotic metabolism at low doses. Brominated isomers are generally more potent inducers of hepatic microsomal enzymes than chlorinated isomers, and the 1,2,4-trihalogenated compound is a more potent inducer than other isomers.

Studies with 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene show these compounds cause enzyme induction, even at low doses. Observations suggest storage in body tissues, with slow release after dose termination, thereby prolonging induction of xenobiotic metabolism.

Numerous studies have shown polychlorinated biphenyls induce xenobiotic metabolism. In view of the results with the halogenated benzenes, similar experiments were conducted to determine the length of time PCBs affect xenobiotic metabolism.

Esterase Activity

Little is known about the ability of halogenated and chlorinated benzenes to alter esterases, except that over 200 pharmacologically active compounds are esters. Therefore hexabromobenzene was compared to less halogenated benzenes regarding the metabolism of acetanilide, which is biotransformed by both an oxidative reaction and an esterase. Hydrolysis of procaine was also studied.

Conclusions

This study increased in scope and depth as research progressed. The interactions affecting xenobiotic metabolism were more complex than originally hypothesized. The more obvious conclusions are stated under subheadings; hepatic prophyria, halogenated aromatics, relationship between induction and storage, and esterase activity.

Hepatic Porphyria

The brominated benzenes did not significantly increase hepatic porphyria. The chlorinated benzenes, other than hexachlorobenzene, had previously been shown to cause minimal increases in liver or urinary porphyrins. Similarly, hexabromobenzene, 1,4-dibromobenzene, and 1,2,4-tribromobenzene (TBB) caused only small increases in hepatic porphyrins, but did not cause increases in ALA synthetase or in the urinary excretion of porphobilinogen, aminolevulinic acid, or porphyrins.

Halogenated Aromatics

The trichlorophenols, which in some species are metabolites or contaminants in halogenated benzenes, were not inducers of xenobiotic metabolism.

The brominated diphenyl ethers were potent short-term inducers of xenobiotic metabolism, as well as potent long-term (90 days) inducers at low doses. Measurements of these ethers in tissue were not made, but it is likely that the extended period of induction, observable 30 to 60 days after the last dose, was a result of their induction potential and accumulation in sites such as adipose tissue and liver.

Chlorinated diphenyl esters were inferior as inducers of xenobiotic metabolism compared to what would be predicted from studies on commercial brominated diphenyl ether mixtures. Diphenyl ether isomers with 2 or 3 chlorines were inactive at the experimental doses. However, similar PCBs exhibited low-induction potential. Pentabromodiphenyl and octabromodiphenyl ether mixtures were more active than 4,4'-dibromodiphenyl ether. The more chlorinated the diphenyl ethers, the better their induction. Curiously, decachlorodiphenyl ether treatment caused a shift in the P-450 peak toward 448, but did not induce AHH; although 2,4,5, 3',4-pentachlorodiphenyl ether caused both effects. The degree of substitution and position of these groups were both important to determine the extent and type of induction.

The toxicity of benzoquinones was very cumulative. A dose far below that of acute toxicity was lethal when given daily for 17 to 30 days. The reasons for this toxicity were not readily apparent. One possibility considered and discarded as erroneous was the occurrence of methemoglobin formation. Both of the chlorinated dibenzofurans induced benzpyrene hydroxylase activity, but dibenzofuran itself did not.

Relationship between Induction and Storage

Both TBB and TCB induced hepatic microsomal enzymes for at least 16 days, after ending 7 days of a 1 mmol per kilogram dose per day. Excretion data indicated more TBB than TCB was held in the body. Metabolism of trihalogenated benzenes to trihalogenated phenols has been demonstrated in rabbits, but these were not inducers of xenobiotic metabolism in rats. The major metabolites in rats appeared to be mercapturic acid derivatives.

Halogenated benzenes were observed in tissues. Increased induction resulting from 4 days of starvation, at 6 days after TBB administration was terminated, suggested TBB was mobilized from fat depots. Phenobarbital treatment immediately after halogenated benzene dosing appeared to hasten mobilization, metabolism, and excretion of either compound. This left smaller amounts in the rat to cause induction at the time of assay.

It was clear that both TBB and TCB were retained in the body and released slowly after administration. On an equimolar basis, TBB led to higher levels of hepatic enzyme induction. These levels were maintained for longer periods and retained to a greater extent in body tissues. TBB was influenced more by starvation than TCB. Starvation accelerated excretion of halogenated benzene. For phenobarbital treatment, this effect was inferred from the decrease in enzyme levels, compared to those in non-phenobarbital-induced animals. A similar relationship of time and loss of elevated xenobiotic metabolism was seen for polychlorinated biphenyls. This induction was also increased by starvation.

Administration of the commercial polychlorinated biphenyl mixture, Aroclor 1254, resulted in increases in xenobiotic metabolism. This persisted for as long as 4 to 5 months.

Esterase Activity

Halogenated benzenes induced xenobiotic metabolism by increasing esterase activity. This applied to phenyl acetate, acetanilide, and procaine indicating broad induction of oxidative, reductive, conjugative, and esteratic metabolic reactions. The compounds most active in inducing arylesterase activity in the liver were 1,2,4-TCB and 1,2,4-TBB, shown throughout our studies as better inducing isomers.

Trihalogenated benzenes decreased the inhibitory effect of malathion on cholinesterase activity in the brain, and to a lesser degree in red blood cells, but not in liver or plasma. It therefore appeared that halogenated benzenes protect against malathion or malaoxon toxicity by increasing the detoxification rate.

The trihalogenated benzenes decreased the inhibitory effect of paraoxon on cholinesterase activity in the brain and liver, but not in plasma or red blood cells. Thus, it appears that the halogenated benzenes protect against paraoxon and probably parathion toxicity by increasing the detoxification rate of the pesticides.

Results

Basically, xenobiotic metabolism was induced by chlorinated and brominated benzenes. This induction affects both mixed function oxidases and esterases. Porphyria was not caused by chlorinated benzenes, except for hexachlorobenzene.

Administration of 1,2,4-tribromobenzene caused a significant increase in liver weight, even at the lowest level of 50 mg/kg in 30 days. An increase in porphyrin content in the liver did not appear until 90 days (Table 1). The

Table 1. *Effect of 1,2,4-Tribromobenzene PO on Porphyrin Production and Excretion in Female Rats*

Dose (mg/kg)	*Liver wt (g)*	*Liver porphyrins (ng/g)*	*Urine porphyrins (μg/24 h)*
		30 days of administration	
0	6.46 ± 0.34^{a}	526 ± 49^{a}	2.19 ± 0.28^{a}
50	8.94 ± 0.56^{b}	602 ± 90^{a}	2.29 ± 0.69^{a}
100	8.94 ± 0.56^{b}	620 ± 66^{a}	2.40 ± 0.13^{a}
200	9.52 ± 0.30^{b}	471 ± 22^{a}	2.53 ± 0.27^{a}
		60 days of administration	
0	7.30 ± 0.21^{a}	156 ± 64^{a}	1.92 ± 0.58^{a}
50	9.16 ± 0.42^{b}	251 ± 75^{a}	1.61 ± 0.21^{a}
100	9.96 ± 0.37^{b}	285 ± 78^{a}	1.77 ± 0.48^{a}
200	9.72 ± 0.50^{b}	2.85 ± 54^{a}	2.42 ± 0.54^{a}
		90 days of administration	
0	6.44 ± 0.26^{a}	468 ± 12^{a}	$1.41 \pm 0.23^{a,b}$
50	8.97 ± 0.43^{b}	629 ± 20^{b}	1.17 ± 0.18^{a}
100	10.07 ± 0.18^{b}	700 ± 17^{c}	2.43 ± 0.59^{b}
200	12.20 ± 0.88^{c}	711 ± 26^{c}	$1.88 \pm 0.35^{a,b}$
		120 days of administration	
0	7.27 ± 0.39^{a}	411 ± 13^{a}	1.75 ± 0.39^{a}
50	11.11 ± 0.80^{b}	560 ± 33^{b}	1.81 ± 0.42^{a}
100	12.71 ± 0.84^{b}	$646 \pm 28^{b,c}$	2.27 ± 0.56^{a}
200	15.18 ± 0.23^{c}	682 ± 45^{c}	2.13 ± 0.40^{a}

$^{a-c}$*Values with same superscript are not significantly different ($p>0.05$).*

increases were dose dependent, small and very similar to those previously observed with 1,2,4-trichlorobenzene.

Animals treated with 200 mg/kg of 1,2,4-tribromobenzene for 120 days were slightly smaller than the controls, but not statistically significant. They were discolored and their ears brown and ragged in appearance. Thus, although the animals looked unhealthy, porphyria was not the problem.

Hexabromobenzene was not shown to share the extreme porphyrinogenic properties of its chlorinated analog. The less brominated compounds were similar to the chlorinated analogs in being very weak porphyria inducers.

Halogenated Aromatics

Chlorinated Phenols—

All of the experimentation resulted in an excess of negative data for halogenated aromatic induction of xenobiotic metabolism. These compounds did not induce EPN detoxification, NADPH cytochrome *c* reductase, or cytochrome P-450. Specific compounds, such as 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene, did not depend upon biotransformation to trichlorophenols for induction ability, so they must be regarded as inactive metabolites.

In vitro the trichlorophenols were inhibitors of EPN detoxification and *p*-nitroanisole demethylation (Table 2). For *p*-nitroanisole demethylation, the inhibition was noncompetitive. The 2,3,5- and 2,4,5-isomers were the most potent.

Brominated Diphenyl Ethers—

The brominated diphenyl ethers, including the complex commercial mixtures of pentabromodiphenyl and octabromodiphenyl ethers, (Table 3), caused liver enlargement (Table 4). Pentabromodiphenyl ether gave the largest increase of 64%. A similar pattern was seen with both NADPH cytochrome *c* reductase and cytochrome P-450 using pentabromodiphenyl ether. The effect of pentabromodiphenyl ether was greater than that of octabromodiphenyl ether, which was equal to bis(*p*-bromodiphenyl) ether. Decabromodiphenyl ether was without effect. This was similar to the poor induction properties of fully brominated benzene.

When a series of enzyme activities were determined, pentabromodiphenyl ether and octobromodiphenyl ether were the more potent inducers, giving large increases in both EPN detoxification and *p*-nitroanisole demethylation (Table 5). They were the only compounds which caused increased conjugation of naphthol. Benzo(a)pyrene hydroxylase was increased only by pentabromodiphenyl ether.

Administration of 6.25, 12.5 or 25 μmol/kg daily of the pentabromodiphenyl ether and octabromodiphenyl ether over a 90-day period resulted in large increases in EPN detoxification, *p*-nitroanisole demethylation, and cytochrome P-450 (Table 6). Pentabromodiphenyl ether did not cause large

Table 2. Effect of Trichlorophenols In Vitro *on p-Nitroanisole O-demethylation and EPN Detoxification*

Treatment	*p-Nitroanisole O-demethylation*[c] *(μg p-nitrophenol/ 50 mg/30 min)*	*EPN detoxification*[c] *(μg p-nitrophenol/ 50 mg/30 min)*
Control[a]	6.64 ± 0.68	8.39 ± 0.34
2,3,5-Trichlorophenol[b]	0.57 ± 0.21[d]	4.02 ± 0.49[d]
2,3,6-Trichlorophenol[b]	2.02 ± 0.22[d]	4.58 ± 1.14[d]
2,4,5-Trichlorophenol[b]	0.61 ± 0.24[d]	3.88 ± 0.47[d]
2,4,6-Trichlorophenol[b]	2.33 ± 0.19[d]	4.03 ± 1.17[d]

[a] *50 μl benzene added.*
[b] *Compound added in 50 μl benzene to give final concentration of 2.5 x 10^{-4} M.*
[c] *Mean ± S.E. of 4 experiments.*
[d] *Significantly different from control ($p<0.05$).*

Table 3. Composition of Bromodiphenyl Ethers

Compound	Percent of bromodiphenyl 0	2	4	5	6	7	8	9	10	Mol. wt. or conglomerate Mol. wt.
Diphenyl ether	98									170
Bis (p-bromophenyl) ether	[a]									328
Pentabromodiphenyl ether			24.6	58.1	13.3	2.6	0.3	0.2	0.8	564
Octabromodiphenyl ether				1.1	8.5	45.1	30.7	13.0	1.6	766
Decabromodiphenyl ether									[b]	959

[a] *Reagent grade.*
[b] *High purity.*

Table 4. Effect of Bromodiphenyl Ethers on Liver wt./Body wt., NADPH Cytochrome c Reductase and Cytochrome P-450

Treatment[a]	$\frac{\text{Liver wt.}}{\text{Body wt.}} \cdot 100$[b]	*Cytochrome c reductase, nmol Cyto. c reduced/ min/mg protein*[b]	*Cytochrome P-450 nmol/mg protein*[b]
Control	3.80 ± 0.12[c]	151 ± 26[c]	0.79 ± 0.05[c]
Diphenyl ether	4.03 ± 0.13[c,d]	134 ± 25[c]	0.71 ± 0.04[c]
Bis (p-bromophenyl) ether	4.48 ± 0.12[d,e]	238 ± 13[d,e]	1.61 ± 0.09[d]
Pentabromodiphenyl ether	6.25 ± 0.25[f]	315 ± 20[f]	2.56 ± 0.17[e]
Octabromodiphenyl ether	5.54 ± 0.25[g]	281 ± 34[e,f]	1.86 ± 0.14[d]
Decabromodiphenyl ether	4.75 ± 0.10[e]	175 ± 5[c,d]	0.91 ± 0.05[c]

[a] *0.1 mmol/kg/day for 14 days po. Controls received corn oil.*
[b] *Mean ± S.E. for 4 rats.*
[c–g] *Values with the same superscript are not significantly different from one another ($p>0.05$).*

Table 5. *Effect of Bromodiphenyl Ethers on EPN Detoxification, p-Nitroanisole Demethylation, Benzo(a)pyrene Hydroxylase, and UDP-Glucuronyltransferase*

Treatment[a]	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min[b]	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min[b]	UDP-Glucuronyl-transferase, nmol napthol/ mg protein/min[b]	Benzo(a)pyrene hydroxylase, nmol/mg protein/10 min[b]
Control	6.60 ± 1.12[c]	6.33 ± 0.78[c,d]	6.23 ± 1.25[c]	6.79 ± 0.54[c]
Diphenyl ether	11.08 ± 1.72[d]	5.68 ± 0.85[c]	5.76 ± 0.73[c]	4.44 ± 0.55[c]
Bis (p-bromophenyl) ether	10.42 ± 1.24[c,d]	9.25 ± 0.75[d]	7.65 ± 1.12[c,d]	4.86 ± 0.74[c]
Pentabromodiphenyl ether	15.98 ± 1.22[e]	43.81 ± 1.93[e]	10.87 ± 1.74[d]	11.01 ± 1.07[d]
Octabromodiphenyl ether	16.30 ± 1.17[e]	17.84 ± 0.41[f]	10.36 ± 1.14[d]	6.01 ± 1.06[c]
Decabromodiphenyl ether	7.10 ± 0.85[c]	8.68 ± 1.06[c,d]	5.97 ± 0.98[c]	5.52 ± 0.61[c]

[a]0.1 mmol/kg/day for 14 days po. Controls received corn oil.
[b]Mean ± S.E. of 4 rats except: 3 rats in pentabromodiphenyl ether group in liver wt./body wt. and glucuronyltransferase and 2 for that group in benzo(a)pyrene hydroxylase.
[c-f]Values with the same superscript are not significantly different from one another ($p > 0.05$).

Table 6. *Effect of Pentabromodiphenyl Ether and Octabromodiphenyl Ether on EPN Detoxification, p-Nitroanisole Demethylation, Cytochrome c Reductase Activity, and Cytochrome P-450 Content*

Dose µmol/kg/day for 90 days	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min	Cytochrome c reductase, nmol Cyto. c reduced/ min/mg protein	Cytochrome P-450 nmol/mg protein
		Pentabromodiphenyl ether		
0	9.6 ± 0.3[a]	7.6 ± 0.1[a]	88 ± 6[a]	0.60 ± 0.04[a]
6.25	17.7 ± 0.5[b]	25.6 ± 1.2[b]	117 ± 9[b]	1.02 ± 0.06[b]
12.5	21.5 ± 0.6[c]	34.6 ± 1.1[c]	120 ± 5[b]	1.16 ± 0.07[b]
25	26.5 ± 0.7[d]	50.1 ± 1.3[d]	138 ± 7[b]	1.14 ± 0.07[b]
		Octabromodiphenyl ether		
0	9.7 ± 0.5[a]	10.0 ± 0.4[a]	171 ± 11[a]	1.04 ± 0.10[a]
6.25	21.3 ± 1.1[b]	20.9 ± 1.8[b]	197 ± 9[a]	1.56 ± 0.13[b]
12.5	26.9 ± 2.2[b]	27.3 ± 1.3[c]	196 ± 18[a]	1.27 ± 0.16[a,b]
25	37.1 ± 2.0[c]	38.4 ± 1.9[d]	208 ± 11[a]	1.46 ± 0.09[b]

[a-d]Values with same superscript are not significantly different from one another ($p > 0.05$).

increases in NADPH cytochrome *c* reductase, nor did octabromodiphenyl ether even at the highest dose level.

When administered for 90 days, even the lowest dose of pentabromodiphenyl ether (0.78 µmol/kg daily) caused increases in EPN detoxification, *p*-nitroanisole demethylation, NADPH cytochrome *c* reductase, and cytochrome P-450 (Table 7). For EPN detoxification and *p*-nitroanisole demethylation, there was a clear-cut dose response relationship.

Chlorinated Diphenyl Ethers—

Chlorinated isomers proved to be poor inducers of xenobiotic metabolism when compared to the very potent induction ability of the polybrominated diphenyl ethers. Only two of the isomers caused increases in aryl hydrocarbon hydroxylase, 3,4,2,4-tetrachlorodiphenyl ether and 2,4,5,3',4'-pentachlorodiphenyl ether.

When microsomal NADPH cytochrome *c* reductase activity was measured, only decachlorodiphenyl ether caused an increase. This compound caused the largest increase in cytochrome P-450 content. Small but significant elevations were also observed in rats treated with both 2,4,5,2',4',- and 2,4,5,3',4'-pentachlorodiphenyl ether.

Dibenzofurans—

Dibenzofuran and its chlorinated derivatives did not increase EPN detoxification or NADPH cytochrome *c* reductase. Benzypyrene hydroxylase activity was induced by both chlorinated dibenzofurans, but not by the parent compound. Both chlorinated dibenzofurans increased cytochrome P-450 content, although the increase was not statistically significant with the 2,8-dichloro isomer. Both also caused a shift to 448.2 nm for the peak of cytochrome P-450 absorbance. These factors strongly support the contention that the compounds are inducers of the 3-methylcholanthrene type.

Relationship between Storage and Induction

Halogenated Benzenes—

Due to the large difference in molecular weight of the halogenated benzenes tested previously they were compared on a molar basis. A dose of 1 mmol/kg/day for 7 days was used in all studies. This dose caused a high level of induction on day 1 after the 7 days with both TBB and TCB (Figures 1 and 2). EPN detoxification levels were approximately twice as high as control values 16 days after

the last dose of TBB, suggesting induction may have occurred beyond day 16 of recovery. The decline in induction was slower with TBB than TCB.

Duration of Induction by PCB and Influence of Starvation—

The results of Aroclor 1254 administration were as expected. EPN detoxification and *p*-nitroanisole demethylation were elevated four- and ten-fold, respectively, 1 day after the last (14th) dose.

Measurements of microsomal NADPH cytochrome *c* reductase and cytochrome P-450 revealed similar results. Cytochrome P-450 increases were observed for the first 71 days, due to Aroclor alone, before returning to control levels at later time periods. Changes in NADPH cytochrome *c* reductase, although not as large, followed a similar time course.

Esterase Activity

Acetanilide and Procaine Esterase—

Halogenated benzenes induced the metabolism of acetanilide and procaine by increasing esterase activity. This was true of the less halogenated benzenes, as well as hexachlorobenzene. These compounds were broad inducers of oxidative, reductive, conjugative, and esteratic metabolic reactions.

Esterases and Pesticide Toxicity

The 1,4-dihalogenated benzenes and hexabromobenzene had little effect on the lethality of malathion. Both trihalogenated benzenes increased the LD50 values, which indicated a decrease in lethality. The trihalogenated benzenes also decreased the toxicity of the active metabolite of malathion, malaoxon, approximately two-fold. Even greater protection against lethality was demonstrated with parathion.

The effect of the trihalogenated benzenes on cholinesterase activity in the brain, liver, plasma, and red blood cells was measured, since toxicity of the organophosphates is related to inhibition of cholinesterase. Malathion caused a decrease in cholinesterase activity in the brains of control mice, but not in those treated with 1,2,4-trichlorobenzene. The decreases were similar between control and treated groups in other tissues. Similarly, 1,2,4-tribromobenzene treatment prevented the decrease in brain cholinesterase following malathion treatment, and slightly decreased the malathion effect on red blood cells.

Measurement of carboxylesterase activity with malathion as the substrate revealed that 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, 1,4-dibromobenzene, 1,2,4-tribromobenzene, and hexabromobenzene all increased carboxylesterase activity in the liver. A similar trend was seen in plasma. The trihalogenated benzenes caused the largest increases in activity.

Paraoxon dearylation was examined since halogenated benzenes might protect against paraoxon toxicity by increasing its metabolism. Plasma esterase was not altered by 1,4-dihalogenated or hexahalogenated isomers. Trihalogenated isomers actually decreased this activity. As expected, there was little activity in either control or treated animals in the 105,000 x g liver supernatant. Microsomal esterase activity was increased by 1,2,4-trichlorobenzene, hexachlorobenzene, and hexabromobenzene, but was inhibited by 1,4-dibromobenzene and 1,2,4-tribromobenzene. However, the mixed function oxidase dearylation in liver microsomes was increased eightfold by 1,2,4-tribromobenzene and to a lesser extent by 1,4-dichlorobenzene, hexachlorobenzene and 1,2,4-trichlorobenzene.

Arylesterase—

None of the halogenated benzenes tested caused any increase in serum arylesterase activity. These compounds included 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachlorobenzene, 1,2,4-tribromobenzene, 1,3,5-tribromobenzene, and hexabromobenzene. Increased activity was observed in the liver after 1,2,4-trichlorobenzene, hexachlorobenzene, 1,2,4-tribromobenzene, and 1,3,5-tribromobenzene. The compounds, therefore, associated throughout this research project as the better

Table 7. Effect of Pentabromodiphenyl Ether on EPN Detoxification, p-Nitroanisole Demethylation, NADPH Cytochrome c Reductase, and Cytochrome P-450

Dose µmol/kg/day for 90 days	EPN detoxification, µg p-nitrophenol 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol 50 mg/30 min	NADPH Cytochrome c reductase, nmol Cyto. c reduced min/mg protein	Cytochrome P-450, nmol/mg protein
		Administration		
0	5.8 ± 0.1^{a}	5.5 ± 0.4^{a}	165 ± 10^{a}	1.05 ± 0.02^{a}
0.78	8.1 ± 0.2^{b}	9.7 ± 0.6^{b}	215 ± 13^{b}	1.41 ± 0.07^{b}
1.56	8.9 ± 0.3^{b}	12.2 ± 0.8^{c}	$194 \pm 21^{a,b}$	1.26 ± 0.06^{b}
3.13	11.4 ± 0.6^{c}	16.9 ± 0.4^{d}	$209 \pm 14^{a,b}$	1.25 ± 0.09^{b}
		Administration plus a 30-day recovery period		
0	6.0 ± 0.2^{a}	5.5 ± 0.4^{a}	128 ± 9^{a}	0.80 ± 0.07^{a}
0.78	7.2 ± 0.2^{b}	7.5 ± 0.3^{b}	$138 \pm 1^{a,b}$	0.87 ± 0.04^{a}
1.56	7.4 ± 0.5^{b}	7.9 ± 0.4^{b}	$138 \pm 8^{a,b}$	0.96 ± 0.18^{a}
3.13	9.1 ± 0.2^{c}	10.7 ± 0.9^{c}	152 ± 6^{b}	0.90 ± 0.13^{a}
		Administration plus a 60-day recovery period		
0	6.3 ± 0.3^{a}	3.6 ± 0.3^{a}	155 ± 9^{a}	0.82 ± 0.07^{a}
0.78	6.4 ± 0.3^{a}	3.8 ± 0.2^{a}	162 ± 14^{a}	0.85 ± 0.12^{a}
1.56	7.7 ± 0.3^{b}	4.6 ± 1.0^{b}	148 ± 7^{a}	0.85 ± 0.06^{a}
3.13	7.5 ± 0.4^{b}	5.6 ± 0.3^{c}	172 ± 8^{a}	0.82 ± 0.06^{a}

[a-d]For each time period, values with same superscript are not significantly different from one another ($p > 0.05$).

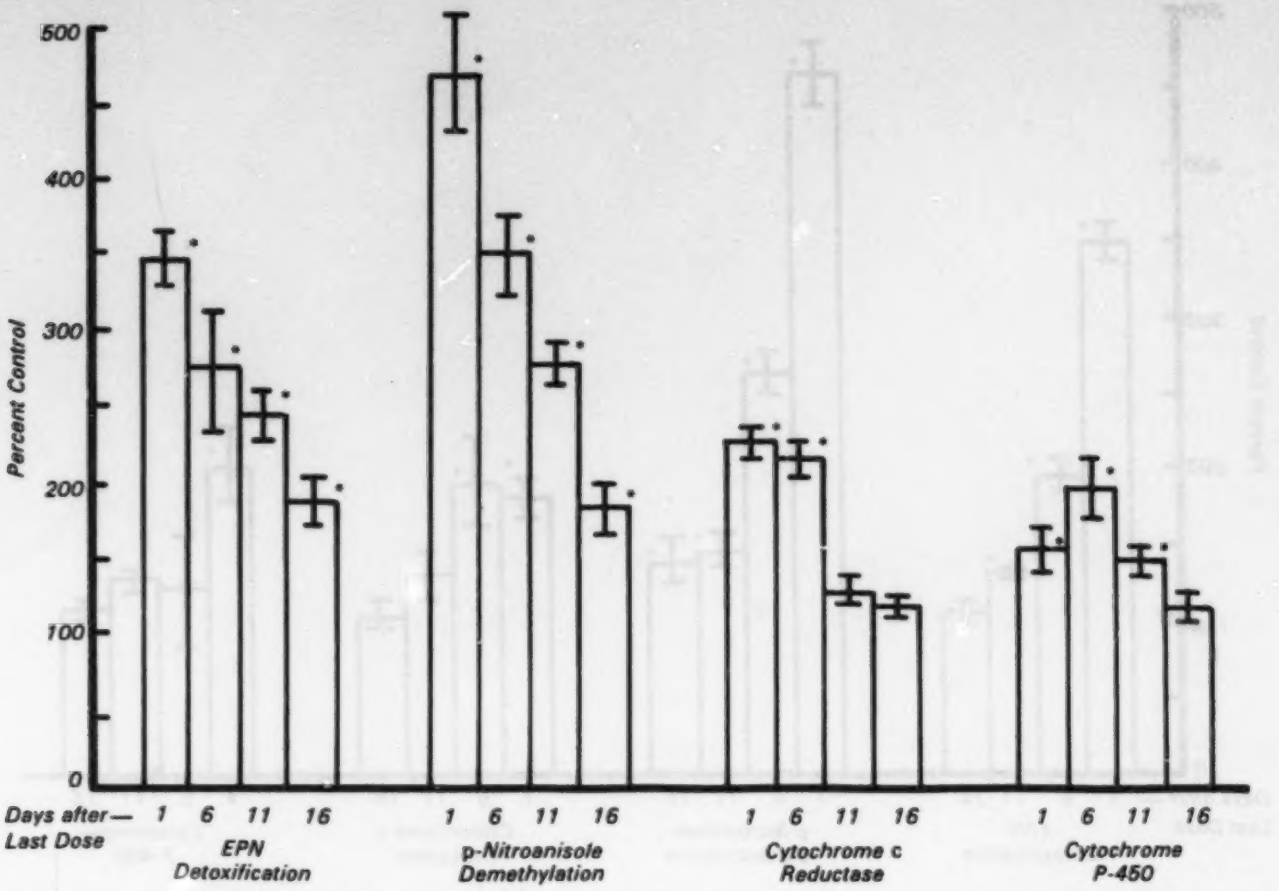

Figure 1. Decline with time in rats receiving TBB (1 mmol/kg/day for 7 days po. Asterisk indicates values significantly different from controls (P 0.05). Average control values: EPN detoxification, 5.9 ± 0.2 μg p-nitrophenol/50 mg/30min; p-nitroanisole demethylation, 5.1 + 0.3 μg p-nitrophenol/50mg/30 min; NADPH-cytochrome c reductase, 118 ± 6 nmol cytochrome c reduced/min/ml protein; P-450, 0.85 ± 0.07 nmol/mg protein.

inducing isomers, were also the most active in inducing arylesterase activity in the liver.

Recommendations

The results of these studies support our earlier findings that many of the halogenated aromatic compounds are capable of altering the metabolism of foreign organic compounds. These reactions include the mixed function oxidases, conjugative enzymes, and esterases. A critical area in need of additional research is the relationship of storage to prolonged induction.

In our studies on halogenated benzenes and PCBs, the period of increased xenobiotic metabolism lasted far beyond the period of administration of the compounds. The halogenated benzenes were able to correlate this activity with storage, especially in adipose tissue. This is also suggested, but not proven, for PCBs. More studies on PCBs and brominated diphenyl ethers are needed.

The chlorinated benzofurans were found to be inducers, supporting previous findings. The chlorinated benzoquinones did not prove to be inducers, but did show highly cumulative toxicity. The reasons for this are not known, but are evidently not related to methemoglobin formation or liver damage. The dramatic nature of this cumulative toxicity indicates subsequent study is needed to ascertain the mechanism involved.

The brominated diphenyl esters were among the most potent inducers tested in our laboratory. Little is known about their pharmacokinetic properties or effects, other than those associated with xenobiotic metabolism. Additional studies are needed to quantify the tissue distribution, metabolism, and rate of elimination. These complex mixtures of isomers need to be identified to ascertain the active ones causing the induction process.

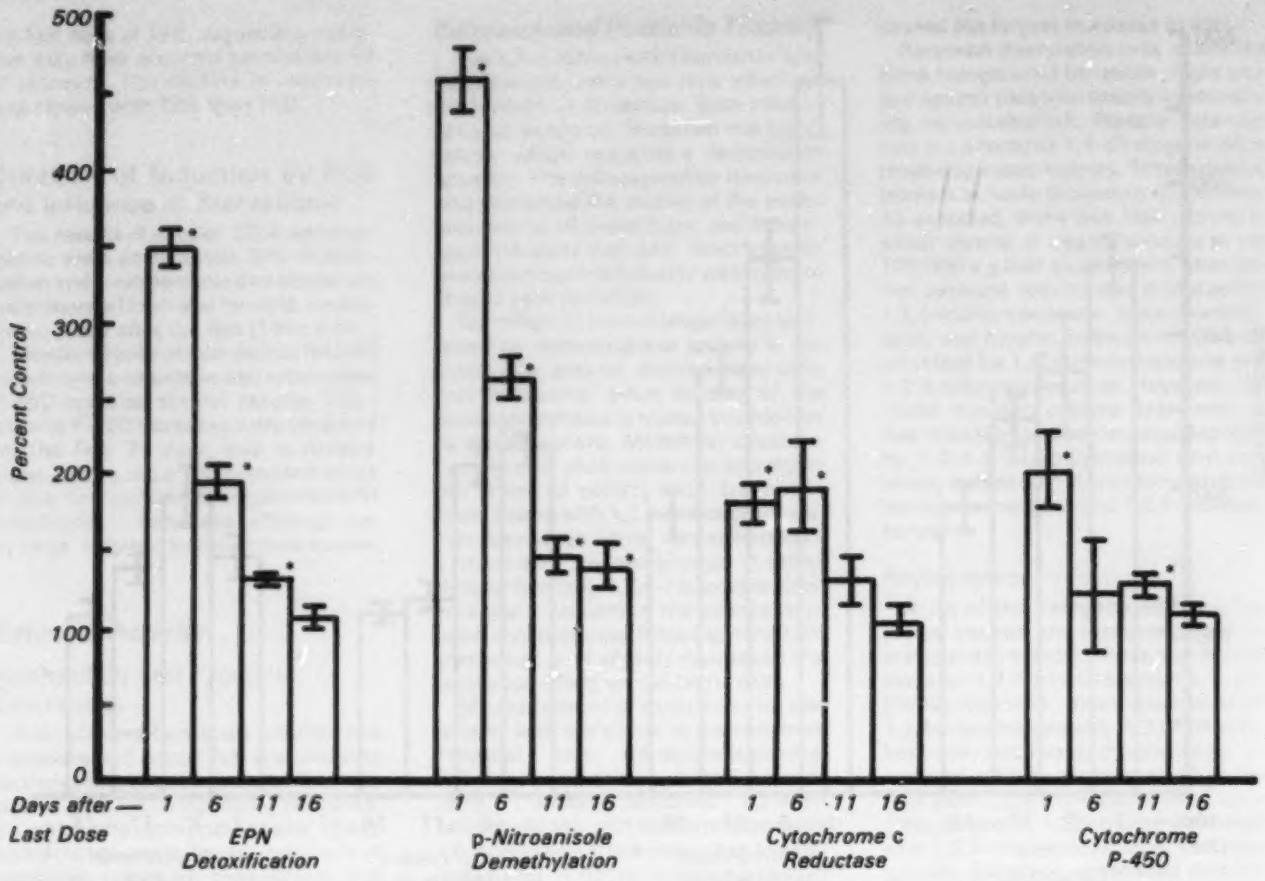

Figure 2. *Decline with time in rats receiving TCB. Average control values: EPN detoxification, 6.3 ± 0.4 μg/50 mg/30 min; p-nitroanisole demethylation, 5.1 ± 0.2 μg/50 mg/30 min; NADPH-cytochrome c reductase, 118 + 6 nmol/min/mg; P-450, 0.85 + 0.07 nmol/mg.*

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***Merrel Robinson** is the EPA Project Officer (see below).*
The complete report, entitled "Effects of Halogenated Aromatic Compounds on the Metabolism of Foreign Organic Compounds," (Order No. PB 81-152 522; Cost: $9.50, subject to change) will be available only from:
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